THE PROTEIN POISON.

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For many years I have been studying the chemistry of the bacterial cell. In 1900 I devised the large tanks for growing massive cultures. These have proved quite satisfactory, and I have been able to get bacterial cellular substances quite free from all impurities, in large amount. After many years of unsuccessful effort Wheeler and I, in 1903, succeeded in partially isolating the poisonous group from the cellular substance of certain pathogenic bacteria. This we did by heating the cellular substance with a two per cent. solution of sodium hydroxid in absolute alcohol. When this is done at the temperature of boiling alcohol the cell substance is split up into a poisonous and a non-poisonous part. The former is soluble in alcohol, while the latter is insoluble. This gives us not only a method of preparation, but also one of partial separation. I may say that the evidence that a distinct cleavage of the bacterial cell is secured is shown by the fact that all the carbohydrate and all the phosphorus in this cellular substance remains in the insoulble or non-poisonous part. The poisonous portion contains no phosphorus and no carbohydrate, but it does give the biuret and the Millon reaction, and must therefore be classed as a protein. This protein has never been obtained as yet in a state of chemical purity. The best preparation that we have been able to secure up to this time kills guinea pigs when injected intravenously, in doses of .5 of a mg. There are certain reasons for believing that its effect upon man is still more pronounced.

Having found this poison in pathogenic bacteria we next looked for it in non-pathogenic organisms, and we found it in these quite as abundantly as in the pathogenic forms. It therefore follows that the pathogenicity of the bacterial cell does not depend upon

its capability of producing a poison, because all bacterial cells contain a poison. Whether a germ is pathogenic to a given species of animal or not depends upon its capability of growing and multiplying in that animal's body.

Next we looked for this poison in certain animal proteins, such as the white of egg, the proteins of blood serum, the casein of milk, etc. In all of these the same or a like poison was found by the same method. Later we tested vegetable proteins, such as the gluten of flour, the zein of corn meal, the edestin of hemp seed, etc. Up to the present time we have examined more than thirty proteins of bacterial, animal and vegetable origin, and in all of these the same poisonous group has been detected.

It has long been suspected, and indeed I may say, known, that the protein molecule contains a poisonous group. At first it was supposed that the diverse proteins which man takes in his food are but slightly altered in the alimentary canal, and before absorption. It is now known that this is not true, and that in the healthy man all proteins are broken down into amino acids by the ferments of the alimentary canal, and that these amino acids are, either during absorption or directly thereafter, resynthesized so as to form the proteins which are characteristic of man's body. The precipitin test has demonstrated that every species of animal has its own specific protein bodies. Every albuminous molecule contains a poisonous group. Peptones injected into the blood act as poisons; therefore the peptone group contains a poisonous molecule, and it is this poisonous molecule in the peptone group which we have succeeded in partially isolating. The symptoms induced by this protein poison are marked and characteristic. They divide themselves into three distinct groups. Soon after the injection of a minimum fatal dose in one of the lower animals there is evidence of peripheral irritation. This is shown by the fact that the animal becomes restless and attempts to scratch itself, not only the part adjacent to the point of injection, but every portion of its body which it can reach. This is known as the stage of peripheral irritation. In man it is characterized by itching and by an erythematous eruption which begins about the place of injection, and rapidly spreads over the

body. In the second stage the animal lies in a lethargic condition, with rapid, difficult respiration. It prefers not to move, and when urged to do so it shows that it has partially lost the power of coördinating its movements. It drags its posterior extremities, or it sways from side to side. This is known as the paralytic stage. The third stage manifests itself by clonic covulsions, which repeat themselves after intervals of rest, becoming more and more violent, until death results. After reaching the convulsive stage recovery is rare, although it does occasionally occur. The symptoms are produced by the injection of protein poison, whether obtained from bacterial, animal or vegetable proteins. It should be stated that in order to study these symptoms properly the dose should approach the minimum quantity. When the dose is excessive the first and even the second stage may not be observed. The animal is speedily thrown into a convulsion, and death results within a few minutes. When a non-fatal dose is given the first and second stages appear, and may last in guinea pigs for an hour, possibly two, but recovery is rapid and apparently complete. It is of importance to note this fact, that when recovery does take place it follows rapidly, and apparently the animal is as well as ever within two or three hours, and possibly earlier.

We had studied this protein poison and its effects upon animals when the phenomenon of protein sensitization, improperly called anaphylaxis, was discovered. All will understand that protein sensitization is demonstrated by injecting a protein, any protein, into an animal and waiting for a certain length of time, or until the animal becomes sensitized, when a second injection into the same animal causes the symptoms which I have described, in the same order as observed when the protein poison is administered, and that the final effects are the same. Comparing the phenomena of protein sensitization with those of protein poisoning Wheeler and I in 1907 offered the following explanation of protein sensitization: When a foreign protein is injected into an animal it must be disposed of in some way. Unless introduced in large amount it is not eliminated by the kidney. It soon disappears from the circulating blood and is deposited in various tissues, the exact place of deposition depending

upon the kind of protein injected and the species of animal. In order to deal with this foreign material certain body cells develop a specific proteolytic ferment, which splits up the protein injected, and no other. The first dose is gradually split up, and consequently produces no recognizable effect upon the animal. When a proper interval of time is allowed to elapse before the second injection, this new ferment, in the form of a zymogen is stored up in certain cells of the body, and when the second injection of the same protein is made this zymogen is activated, and converted into a ferment which splits up the injected protein with great rapidity, setting free the same poison which we obtained by splitting up proteins with sodium hydroxid in absolute alcohol. This explanation of the phenomena of protein sensitization was published by Wheeler and myself in 1907. Recently it has been confirmed in France by Nicolle and Abt, and in Germany by Friedberger. It is true that Friedberger does not fully give us credit for this work. He says that we suggested this explanation, and he has demonstrated it. It is unfortunately true that much of the scientific work done in America must go to Germany and be approved before it is accepted by other Americans. This is due to our lack of confidence in ourselves and in one another. The German has so long been accustomed to stamp his products as "made in Germany," that much of our scientific work comes back with this stamp upon it. However, it is not my purpose to complain about this matter. My European confreres have given me, on the whole, fair credit for work done along this line.

More recently we have attempted to use the knowledge which we have gained in the study of the protein poison in the explanation of many of the phenomena of immunity and of disease. The essential difference between egg-white and the typhoid bacillus is that the former is a non-labile, dead protein, while the bacillus is a labile, living protein. If egg-white could grow and multiply after being introduced under the skin, or into the blood of an animal, it would be just as dangerous to prick a finger with a needle moistened with this relatively harmless, bland protein as it would to inoculate oneself with the anthrax bacillus. As early as 1907 Wheeler and I held

that protein sensitization and bacterial immunity are one and the same thing. In sensitization the animal dies on the second dose. In immunity the animal survives the second dose. Sensitization and immunity are therefore apparently antipodal, but are in fact the same thing. A man drinks water containing the typhoid bacillus, and he does not develop typhoid fever that day, nor the next. He passes through a period of incubation, which in typhoid fever is somewhere about eight or ten days. During this time the typhoid bacillus is multiplying in his body in great numbers, and in doing so it is converting his proteins into typhoid proteins. Suddenly the period of incubation stops and the disease begins to manifest itself. The period of incubation stops when the body cells have become sensitized to the typhoid protein, and begin to break it up. From that time on the fight is between the living cells of the body with the ferment which they pour out, and the bacilli.

It occurred to us that if this theory be true we might demonstrate it by repeated injections of small quantities of some protein body, and determine what effect such injections might have upon body temperature. In these experiments we have used egg-white principally because we wanted to get away from cellular structure and from the supposed influence of life. We wanted to take a dead substance. Of course in doing so we recognized the fact that egg-white does not grow and multiply in the body, and consequently we must keep up the supply by repeated injections. I have published several papers upon this, notably in the *Zeitschrift für Immunitätsforschung,* and therefore I am relieved from the necessity of going into detail in this article. Suffice it to say that by varying the size of the dose and the interval between the doses one can induce in the lower animals any kind of fever that one wishes. One can place an animal in a typhoid condition, and by repeated injections keep the animal in this condition with a temperature identical with that of typhoid fever for days and weeks. On the other hand, by more frequent injections one can induce in a rabbit an acute, fatal fever, terminating in a few hours; or, by again varying the size of the dose and the interval, one can secure at will the picture of remittent or intermittent fever. Fever, therefore, results from the introduc-

tion of a foreign protein into the body, the sensitization of the body cells to that protein, and finally the cleavage of that protein by the ferment elaborated by the sensitized body cells. Now in nature practically all the proteins that find their way into the body undigested are living proteins, in the form of bacteria or protozoa. They grow and multiply in the body, without materially disturbing for the time being, the life of the individual. This continues during the period of incubation but when the body cells have become sensitized and begin to split up the foreign protein the period of incubation ceases and that of disease begins.

We have shown that repeated injections of foreign protein not only cause fevers of various kinds, but lead to emaciation of the animal body, to increased elimination of nitrogen, and to decreased urinary secretion, and, in short, to all the phenomena that are characteristic of the febrile diseases. Death from any of the infectious diseases is due to one and the same poison, and that poison is a constituent of the protein molecule. Symptoms vary in different diseases for two reasons: In the first place, the foreign proteins have different predilection places in the body in which they are deposited. In the second place the ferment which splits up these foreign proteins is specific for different diseases. The most successful diagnostician cannot determine the nature of the bacterial organism which causes the symptoms of meningitis. The symptoms are the same so long as the organ involved is the same. The meningitis may be due to the meningococcus, to the streptococcus, to the typhoid bacillus, or to the tubercle bacillus. Still, the symptoms are the same because the cleavage of the foreign molecule occurs in the same part of the body. Again, every medical man knows how difficult it is to distinguish between typhoid fever and acute general miliary tuberculosis, because in both instances the foreign protein is largely in the blood current. As I have stated, most bacterial proteins have predilection places in which they are deposited. The typhoid bacillus prefers the mesenteric and other glands; the pneumococcus is deposited generally in the lungs, though it may be found in the intestinal walls. The meningococcus finds its favorite place for growth and development in the coverings of the brain. The tubercle bacil-

lus grows most frequently in the lungs, though it has fed upon man for so long a time that it is now able to sustain itself in almost any part of his body.

From what has been said it must follow that fever on the whole is a beneficent process. It is one of the phenomena of the parenteral digestion of proteins. The foreign protein has gotten into the body, is growing and multiplying, and in doing so is utilizing the proteins of man's body. It must be destroyed, and the body cells pour out a ferment which digests the foreign protein. This is nature's way of disposing of the foreign material, and it is apparently about the only way that nature has of doing it. I repeat therefore that fever on the whole is a beneficent process. It is an attempt on the part of nature to get rid of the invading protein. Like many other of nature's processes it may be overdone, and death may result from fever, per se.

That fever does result from a fermentative cleavage is shown not only by the facts which I have already enumerated, but those which we have learned in combating fever. Nearly all, if not all, of the anti-febrile reagents which have been employed in medicine are anti-ferments, and they lower the temperature by retarding the process of protein cleavage. Both natural and acquired immunity, apart from toxic immunity, may be explained by the facts as stated above. In natural immunity the foreign protein is either unable to grow and multiply, and this means that its ferments are unable to split up the proteins of the body, or the ferments of the body split up the invading protein before it has time to grow and multiply. This explains natural immunity, whether it be racial or individual.

Acquired immunity is explained by the fact that the first attack of the disease, or inoculation with a modified virus, develops in the body cells a ferment which is stored up, and which on a second injection of the same protein, acts rapidly, and effectively, and splits up the invading virus. In vaccination for smallpox we use a virus modified by its passage through the cow. This modified smallpox virus develops in the body cells a ferment which is capable of splitting up the smallpox virus, and the next time this individual comes in contact with a smallpox patient, or receives the smallpox virus, it is split up and destroyed before it has time to grow and multiply.

This also explains the beneficial effects that undoubtedly have been obtained by the various vaccines now so widely and often so unintelligently used.

I wish to suggest that the exanthematous diseases may be explained by the fact that the foreign proteins of certain diseases are deposited in the skin, and that this tissue is the site of the destruction of the foreign body. I may say in support of this that we have repeatedly injected egg-white into the ear vein of rabbits. After varying periods of time we have shown by sensitizing animals with blood taken from the heart that the egg-white has wholly disappeared from the circulating blood of the rabbit. Later it can be shown that this egg-white has been deposited in the skin, in the kidney, in the brain, and in various other organs of the rabbit. It seems to me that our work upon the protein poison furnishes us with facts, by means of which we are able to explain many of the phenomena of immunity and of disease.